The Effects of EPA+DHA Supplements on CRP Levels in Patients with Chronic Venous Leg Ulcers: A Pilot Study in Older Adults

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I. Statement of the Problem

Introduction

Chronic venous leg ulcers (CVLUs) are significant clinical problems because they are so challenging to treat and frequently recur. Moreover, CVLUs have high prevalence rates, especially among the elderly. "The most common type of chronic wound among the ambulatory elderly is thought to be the venous leg ulcer" (Margolis, Bilker, Santanna, & Baumgarten, 2002, p. 381). One study reported that "… in people between 65 and 95 years of age, the annual prevalence of venous leg ulcer was 1.69%, whereas the overall incidence rate was 0.76 for men and 1.42 for women. The overall incidence increases with age, but the gender difference dissipates" (Margolis, Bilker, Santanna, & Baumgarten, 2002, p. 385). Additionally, patients with CVLUs are often required to visit healthcare providers on a regular basis for dressing changes, wound debridement and other procedures designed to facilitate healing. It has been estimated that CVLU patients "incur treatment costs of approximately $3 billion per year in the USA" (Raffetto, 2012, p. 61). Thus CVLUs are associated with physical, emotional and financial burdens for patients and their families.

Non-healing wounds have been associated with high levels of sustained inflammation (Keylock & Young, 2010). A study by Wysocki, Staiano-Coico, and Grinnell (1993) reported that matrix metalloproteinases (MMPs), inflammatory markers, were present in the fluid of CVLUs, indicating that high levels of inflammation could be preventing healing. C-reactive protein (CRP) has also been used as a marker of inflammation in chronic wounds. Patients with non-healing burn wounds were found to have higher serum levels of CRP, and lower baseline levels of CRP have been associated with faster healing of diabetic foot ulcers (Tecilazich et al., 2013; van de Goot, et al., 2009). Therefore, measuring CRP levels before and after an

intervention could help determine how successful the intervention is at resolving inflammation. Data from multiple studies suggest that EPA+DHA, found in fish and fish oils, are anti-inflammatory agents (He et al., 2009; Lopez-Garcia et al., 2004). Moreover, higher EPA+DHA levels have been associated with lower CRP levels (Niu, et al., 2006; Bowden, Wilson, Deike, & Gentile, 2009; Saifullah et al., 2007; Theilla et al., 2012). However, only a few studies have quantified CRP levels before and after EPA+DHA supplement use in patients with chronic wounds and none have evaluated their effect on CVLUs specifically. Therefore, additional studies are needed to determine if EPA+DHA supplements have the potential to decrease CRP levels in patients with CVLUs and potentially help these recalcitrant wounds heal more efficiently.

Background

Wound healing is a complex process that involves multiple body systems, including the circulatory and the immune systems (Keylock & Young, 2010). A wound must proceed through four stages for effective and timely healing to occur: hemostasis, inflammation, proliferation, and remodeling. In the first stage, following injury, a clot is formed to prevent bleeding and then the inflammation stage begins to eradicate any bacteria or pathogens that have entered the body through the broken skin. Next, the wound tissues must undergo proliferation that allows re-epithelialization to occur and the wound to decrease in size. The final stage, remodeling, is when tissues from the previous stage are replaced with type I collagen, which is more stable and thus increases the wound's tensile strength (Keylock & Young, 2010). In most circumstances, in otherwise healthy individuals, the wound healing process occurs in a timely manner, but increased age and comorbidities such as cardiovascular disease (CVD) can cause delays in any one of these healing stages or prevent complete healing. Importantly, the pathogenesis of many

of the comorbidities associated with aging (i.e. CVD) involves chronic inflammation (Salvastru, Nedelcu, & Tiplica, 2012).

Although inflammation is needed in the initial wound healing process, high levels of inflammation over time may compromise healing (Keylock & Young, 2010). Therefore, it may be important to measure levels of inflammation in patients with chronic wounds to determine if an intervention designed to curtail inflammation might be indicated. Measuring levels of inflammation can also help determine if a patient is at risk for other inflammatory conditions such as CVD. CRP has been shown to be a valid and reliable marker of inflammation in multiple studies. For example, a study by the American Heart Association confirmed "the prognostic relevance of CRP, a sensitive systemic marker of inflammation, to the risk of CVD in a large, randomly selected cohort of initially healthy middle-aged men" (Koenig et al., 1999, p. 237). Recently, other studies have found that plasma levels of CRP between 3 and 10 μg/ml are associated with increased risk of developing CVD because chronic inflammation plays a key role in plaque formation along arterial walls (Black, Kushner, & Samols, 2004). Two studies also reported that in different types of wounds (i.e. burn wounds, diabetic foot ulcers) CRP levels were elevated in the wounds that were not healing properly (Tecilazich et al., 2013; van de Goot et al., 2009). The collective studies provide evidence that CRP is a valid marker of inflammation and could be used to determine inflammation status in patients with chronic wounds.

If CRP levels are elevated in patients with CVLUs then interventions that lower CRP levels (and thus inflammation) may promote healing. Data from previous studies have suggested that the omega-3 polyunsaturated fatty acids (PUFAs), eicosapenaenoic acid (EPA) and docosahexaenoic acid (DHA) can lower CRP levels. EPA+DHA are found almost exclusively in fish and fish oils (Swanson, Block, & Mousa, 2012). Although the mechanisms of action are not

completely known, higher EPA+DHA levels have been associated with lower levels of proinflammatory cytokines released from endothelial cells (Niu et al., 2006). The health benefits of EPA+DHA have been related primarily to their ability to diminish inflammation; however, most Americans do not consume enough of these particular PUFAs to elicit anti-inflammatory benefits. Interestingly, a sample of people from Japan were found to have lower CRP levels than people living in the United States, Denmark, Spain, Sweden, and Greece, which was linked to the high number of marine products typically found in the Japanese diet (Niu et al., 2006). Therefore, in order to consume enough EPA+DHA to achieve anti-inflammatory effects, people living in Western countries may require an oral supplement.

Purpose

The purpose of this experimental, randomized controlled pilot study was to determine the effects of oral supplements containing EPA+DHA on plasma CRP levels in a sample of older adults with CVLUs over an interval of 56 days.

Significance

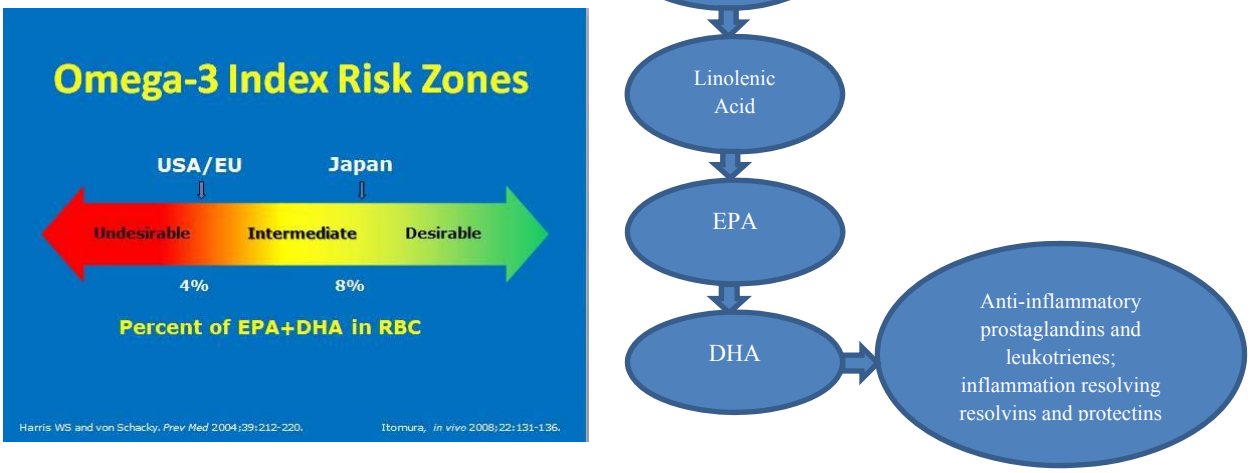
CVLUs create emotional and financial burdens for patients and their families. "Delayed and chronically non-healing wounds can result in increased pain, loss of mobility, and decreased productivity and quality of life" (Keylock & Young, 2010, p. 70). Additionally, "….it has been estimated that venous leg ulcers cause the loss of approximately two million working days" (Raffetto, 2012, p. 61). Patients with CVLUs self-report decreased quality of life, morale, self-esteem, pain, and functional ability (Edwards, Courtney, Finlayson, Shuter, & Lindsay, 2009). CVLUs also create burdens for the clinician because they are so challenging to treat, require repeated visits and frequently recur. Thus, CVLUs negatively affect the patient, the patient's family, healthcare providers and society in general.

Because of the many adverse consequences associated with CVLUs, it is crucial to determine novel adjunct treatment approaches to decrease healing time and prevent their recurrence. An oral supplement containing EPA+DHA may be helpful in reducing CRP levels, so the ulcer can heal in an environment with low inflammation levels. Consuming the supplement would be a simple, non-invasive way to promote wound healing. It would also be important for healthcare providers to encourage patients to maintain a nutritious diet in addition to consuming the EPA+DHA supplements because other nutrients such as vitamin C and zinc are also important for efficient healing. Patients visiting wound clinics on a regular basis have been shown to achieve the best healing outcomes (Jones, 2009). Nurses and other healthcare providers could obtain a dietary assessment on an initial visit, prescribe an EPA+DHA supplement when indicated, and encourage patients to consume a nutrient dense diet to promote healing.

Conceptual Framework of Reference (Theory)

Dr. William S. Harris's theory regarding the inflammation-resolving mechanisms of EPA+DHA guided the study design (Harris & von Schacky, 2004). (Figure 1) Additionally, Dr. Harris collaborated with a researcher from Munich, Dr. Clemens von Schacky, to evaluate the HS-Omega-3 Index. (Figure 1) The test measures the EPA+DHA levels contained in the membranes of red blood cells (RBCs). Data from Dr. Harris's studies have revealed that inflammatory markers were inversely related to the Omega-3 Index and an Omega-3 Index of "≥8% was associated with the greatest cardioprotection" (Harris & von Schacky, 2004, p. 212). His research has also shown that Americans have an Omega-3 index of ~4-5% and that Japanese have an Omega-3 index of ~8%. Groups with higher Omega-3 levels have been found to have less heart disease (Harris & von Schacky, 2004).

Figure 1. Omega-3 index Risk Zones and Omega-3 Metabolic Pathway to Anti-inflammatory Mediators



In a cross-sectional study by Dr. Harris looking at patients with peripheral artery disease (PAD), "the Omega-3 index was inversely associated with plasma levels of CRP and IL-6" (Grenon et al., 2013, p. 1289). The study reported that "because patients with PAD and elevated CRP values are at higher risk for cardiovascular and revascularization outcomes, further studies are needed to determine if manipulation of the Omega-3 index with dietary changes or fish oil supplementation could reduce vascular inflammation and related symptoms" (Grenon et al., 2013, p. 1289). Therefore, it is important to determine if fish oil supplementation can reduce inflammation levels to prevent chronic inflammatory diseases and in the case of CVLUs, facilitate healing.

Research Question

This study was conducted to answer the following research question: Will a group of older adults with CVLUs who consume oral EPA+DHA supplements for 56 days have lower systemic inflammation, as evidenced by lower CRP levels, than a group of older adults with CVLUs who consume a placebo for the same interval of time?

Limitations

The limitations of this study include a small sample size (17 participants), which limits the generalizability of the findings. Moreover, the participants were all from central Ohio, and the majority had incomes below the poverty line (68%). Evidence shows that people from different geographical regions and income levels have variable dietary patterns, thus additional research is needed using a larger, more diverse population of subjects (Baker, Schootman, Barnidge, Kelly, 2006).

II. Review of Literature

CVLUs have been shown to significantly affect patients' lives emotionally, physically, and financially. "Chronic wounds account for at least $1 billion per year in the United States and $7 billion per year worldwide in health care costs" (Margolis, Bilker, Santanna, Baumgarten, 2002, p. 381). Venous leg ulcers affect "…approximately 1% of the general population" (Raffetto, 2012). As these statistics indicate, CVLUs are a significant health care problem. Additional studies are needed to test interventions for facilitating healing and preventing recurrence.

Systemic, prolonged inflammation has been shown to increase the healing time of wounds. "The pathophysiology of dermal abnormalities in CVLUs is reflective of a complex interplay that involves sustained venous hypertension, inflammation, changes in microcirculation, cytokine and matrix metalloproteinase (MMP) activation, resulting in altered cellular function and delayed wound healing" (Raffetto, 2012, p. 61). Research studies have evaluated the effects of inflammation on burn wounds, diabetic ulcers, and CVD, but only a few studies have focused on CVLUs. One study reported that "…modest elevations in serum CRP concentrations significantly predict future coronary events. These observations strengthen the

association between low-grade inflammation and the progression and complications of atherosclerosis" (Koenig et al., 1999, p. 241). Therefore, it may be important to measure serum CRP levels to determine the level of inflammation in patients with CVLUs.

To promote the healing of CVLUs inflammation needs to be reduced. According to one study, "… the dietary intake of long-chain omega-3 PUFAs and fish is inversely associated with concentrations of some inflammatory biomarkers, reflecting lower levels of inflammation and endothelial activation" (He et al., 2009, p. 1238). Therefore, taking an oral supplement containing the omega-3 PUFAs EPA+DHA (the bioactive components of fish oil) could lower CRP levels in patients with CVLUs, and potentially improve healing rates.

Chronic Venous Leg Ulcers and Inflammation

Chronic levels of inflammation prevent the stages of wound healing to progress in an orderly and timely manner. The four primary stages of wound healing are hemostasis/coagulation, inflammation, proliferation, and remodeling (Keylock & Young, 2010). Inflammation is an important stage, but chronically high levels of inflammation prevent healing progression. Data from a recent study revealed that "… the presence of MMPs in CVLU wound fluid lead to a chronic state of inflammation, tissue turnover and a persistent chronic wound" (Raffetto, 2012, p. 64). The data also showed that high levels of an inflammatory biomarker were associated with nonhealing wounds. High levels of inflammation have also been found in non-healing burn wounds and diabetic ulcers. "The excessive presence of the inflammatory mediators, complement and CRP, and the increased infiltration of neutrophils and macrophages in burn wounds up to 46 days after injury implicate a persistent ongoing acute inflammation locally in the burn wound up to weeks after the initial trauma" (van de Goot et al., 2009, p. 274).

This study demonstrated a link between inflammation and delayed wound healing. Further supporting the idea that wounds heal better in a low-inflammatory environment is a study that "…found that stimulating resolution with RvD1 enhanced closure of diabetic wounds and this was also associated with decreased burden of both apoptotic cells and macrophages. Thus, RvD1 could potentially be used as a novel agent to promote wound healing in diabetic patients" (Tang et al., 2013, p. 622). RvD1 is an anti-inflammatory compound generated from EPA+DHA metabolism. Thus, the data suggested that EPA+DHA may help chronic wounds to heal.

EPA and DHA and Inflammation

EPA+DHA are long-chain omega-3 PUFAs found in fatty fish such as salmon and oral fish oil supplements that have anti-inflammatory properties (Calder, 2006). Adequate EPA+DHA intakes have been shown to suppress inflammation in inflammatory related diseases. For example, a study by He et al. reported that the dietary intake of omega-3 PUFAs and nonfried fish was inversely related to CRP and IL-6 (2009). Moreover, a greater intake of PUFAs from marine products was related to a "lower prevalence of high CRP concentrations" among an older Japanese population (Niu et al., 2006, p. 223). These studies suggest that increasing the consumption of EPA+DHA will reduce inflammation, which is the mechanism through which EPA+DHA is believed to prevent CVD. Another study found that "…dietary (omega-3) fatty acids are associated with levels of these biomarkers reflecting lower levels of inflammation and endothelial activation, which might explain in part the effect of these fatty acids in preventing CVD" (Lopez-Garcia et al., 2004, p. 1806).

Data from two other studies conducted on patients going through dialysis also suggested that EPA+DHA supplements lower CRP levels and therefore lower inflammation levels

(Bowden, Wilson, Deike, Gentile, 2009; Saifullah et al., 2007). In one particular study of patients with end-stage renal disease it was found that "consuming 960 mg/d of EPA and 600 mg/d of DHA can lower CRP" (Bowden, Wilson, Deike, Gentile, 2009, p. 508).

Findings from these collective research studies show that EPA+DHA can lower inflammatory markers in healthy patients and in patients with chronic inflammatory diseases, suggesting that EPA+DHA may also lower inflammation in patients with chronic wounds. A recent study reported that adding fish oil to patients' diets in the ICU may prevent pressure ulcers from worsening (indicated by the PUSH score) (Theilla et al., 2012). The study found that the "…slowing in progression was associated with a decrease in the levels of CRP, suggesting the effect was mediated by anti-inflammatory mechanisms" (Theilla et al., 2012, p. e108). Thus the anti-inflammatory actions of EPA+DHA may aid the healing of chronic wounds such as CVLUs.

C-Reactive Protein

"CRP is a phylogenetically highly conserved plasma protein, with homologs in vertebrates and many invertebrates, that participates in the systemic response to inflammation. Its plasma concentration increases during inflammation, a characteristic that has long been employed for clinical purposes" (Black, Kushner, Samols, 2004, p. 48487). Moreover, many studies have reported that higher CRP levels are associated with greater risk of CVD (Koenig et al., 1999; Emerging Risk Factors Collaboration et al., 2012; Ridker, Hennekens, Buring, & Rifai, 2000). For example, one study found that out "…of 12 plasma variables, hs-CRP proved to be the strongest and most significant predictor of the risk of future cardiovascular events" (Ridker, Hennekens, Buring, & Rifai, 2000, p. 842). Thus studies showing that EPA+DHA are inversely related to CRP levels (He et al., 2009; Theilla et al., 2012; Niu et al., 2006; Bowden, Wilson,

Deike, Gentile, 2009) provide support for their use to treat or potentially prevent chronic inflammatory diseases.

Lower CRP levels have also been associated with more efficient healing wounds. One study found that lower baseline levels of CRP were associated with greater healing of diabetic foot ulcers (Tecilazich et al., 2013) and another study suggested that CRP could be used as a predictor of potential wound healing disorders (Blass et al., 2013).

III. Methodology

Research Design

This pilot study was an experimental, randomized controlled trial in which human subjects with CVLUs were studied at baseline and at 56 days following eligibility screening, informed consent, and enrollment to the study. Participants were randomly assigned to the Control Group receiving a placebo or to the Active Group receiving EPA+DHA supplements. Participants assigned to the Active Group were given omega-3 EPA+DHA supplement softgels (five/day) and 81mg aspirin (ASA) tablets (one/day) until the study was complete. Aspirin was given because it enhances the action of the EPA+DHA generated resolvin species (Serhan, Hong, Gronert, et al., 2002). Participants assigned to the Control Group were given placebo softgels that look identical to the EPA+DHA supplements and 81mg ASA tablets, which were to be taken on the same schedule as the Active Group. CRP levels were measured at 0 and 56 days in the Active and Control Groups to determine levels of systemic inflammation before and after treatment with EPA+DHA or placebo. The time points were chosen to allow adequate time for the supplements to affect plasma PUFA levels (McDaniel, Belury, Ahijevych, & Blakely, 2008;

McDaniel, Massey, & Nicolaou, 2011) and, potentially, the CRP levels. Body mass index (BMI) was calculated to evaluate its association with CRP levels.

Population and Sample Design

This pilot study collected data from 17 adults ≥ 18 years of age. Potential subjects were identified by reviewing medical records from a Comprehensive Wound Clinic in Central Ohio. Participants were also recruited through advertisements placed on bulletin boards in the Wound Clinic. Interested individuals were given the study details and were screened for eligibility. After eligibility screening, informed consent, and enrollment to the study the participants were randomly assigned to either the Control Group or the Active Group. The study was approved by a Biomedical Institutional Review Board (IRB) and conducted in compliance with the ethical rules for human experimentation as stated in the 1975 Declaration of Helsinki.

Inclusion Criteria

•Chronic venous leg ulcer between the ankle and knee for at least 3 months with prescribed compression therapy

•Ankle Brachial Pressure Index of at least 0.8

•Ambulatory

•Target wound of at least 1 x 1 inches

•Understand English and have the ability to sign own consent

Rationale for inclusion criteria: A CVLU lasting at least three months is considered to be chronic and compression therapy is the gold standard for treatment. An ankle brachial pressure index of at least 0.8 is indicative of a venous ulcer. Individuals who understand English are more likely to understand the study details contained in the consent form than those who do not and it

was important for the participants to be able to sign their own consent to be sure it was their decision to join the study.

Exclusion Criteria

•allergy to fish or other seafood

•any bone, tendon, or fascia exposed near target wound

•prescribed anti-coagulants

•immunologic related conditions, chronic inflammatory skin disease, or diabetes mellitus

•requiring non-steroidal anti-inflammatory drugs more than two times per week, nutritional supplements, or corticosteroids

•chronic renal insufficiency

•already participating in a study related to CVLU

Rationale for exclusion criteria: Participants could not have an allergy to fish or seafood because the EPA+DHA supplements contain fish oil. If bone, tendon, or fascia is exposed more healing complications exist. Some studies indicate that taking anti-coagulants with fish oil supplements can decrease coagulation times (Buckley, Goff, & Knapp, 2004). Immunological conditions, inflammatory skin diseases, anti-inflammatory drugs, nutritional supplements, corticosteroids, and chronic renal insufficiency may affect outcomes measures. Additional study protocols may conflict.

Setting: The Ohio State University Clinical Research Center (CRC)

Data Collection Procedures

Visit 1: Baseline

At the first visit the study was reviewed with each participant again and time was allowed

for questions before the IRB-approved consent form was presented for signature. Participants' sociodemographic information and body measurements (height and weight) were collected. Body mass index was computed from these measurements. Blood samples were collected to quantify CRP levels. Participants received a pill bottle containing their supply of softgels containing EPA+DHA or placebo and another container containing aspirin tablets (81 mg/d). Participants were instructed to store the softgels in the refrigerator and to take five softgels and one aspirin tablet every day. Participants received instructions to continue their normal diets, except to exclude fish, seafood, algae, kelp, and nutritional supplements until the study was complete. Participants were also instructed to avoid taking non-steroidal anti-inflammatory drugs (NSAIDs), other than the 81 mg/d of aspirin, because NSAIDs may affect CRP levels.

Visit 2: 56 days after baseline

Blood samples were collected again for CRP quantification. BMI was calculated. The empty pill bottles were collected.

Data Collection Instruments

Socio-demographic data: Participants completed a form wherein they self-reported gender, age, income level($0-4,999; $5,000-9,999; $10,000-14,999; $15,000-19,999; $20,000-24,000; $25,000-29,999; $30,000-34,999; $35,000-39,999; $40,000-44,999; $45,000 and up), years of education (less than 7 years, junior high school, some high school, high school, some college, college or university graduate [Bachelors or equivalent], and graduate or professional training [Masters, JD, MD, PhD, etc.]), marital status, employment, smoking status, exercise/activity level, amount of alcoholic drinks in a week, number of cups of caffeinated beverages per day, and race/ethnicity.

Serum hs-CRP Concentrations: Serum hs-CRP concentrations were quantified using the Cayman's CRP (human) EIA Kit, an immunometric assay. The standard curve spans the range of 0-3,000 pg/ml with a limit of detection of approximately 50 pg/ml. The assay is based on a double antibody 'sandwich technique'. In the microwell plate each well is coated with monoclonal antibody specific for human CRP. This antibody binds to human CRP that is introduced to the well. Standards and samples are incubated on the antibody-coated plate and the plate is rinsed and then the HRP-labeled CRP monoclonal antibody to detect captured CRP is added. The two antibodies form a 'sandwich' by binding to different locations on the CRP molecule. The concentration of the analyte is determined by measuring the enzymatic activity of the HRP using the chromogenic substrate TMB. After a sufficient amount of time, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of the color, determined spectrophotoetrically, is directly proportional to the amount of bound HRP-labeled monoclonal antibody, which in turn is proportional to the concentration of the CRP.

Anthropometric Data: Height and weight were collected and used to calculate the BMI by the CRC nurses. Height was measured using the Harpendon Stadiometer (Holtain Limited, Crymych, Dyfed, U.K.) to the nearest 0.1 cm. Body weight was measured using the ProPlus Scale (Healthometer, Bridgeview Illinois) to the nearest 0.1 kg. BMI was calculated as body weight (kg) divided by height (m) squared.

Data Analysis

Descriptive statistics, including percent, range, mean, and standard deviation (SD) were used to characterize socio-demographic, hs-CRP serum concentrations, and anthropometric data.

IV: Results

Participant Characteristics

Data were collected from 17 adults from Central Ohio, ages 28 to 81 years, who had one or more CVLUs for at least 3 months (Table 1). The mean age of the total sample was 62 ±12.51 years, containing 10 males and 7 females. The majority of participants self-reported White as their race (82%) and 10 of the 17 participants self-reported an income of <$19,999/year ($15,930 is poverty line for 2-person household). The majority of participants were either a high school graduate (35.3%) or a university graduate (35.3%), with 29.4% having some college. Additionally, the average body mass index (BMI) was 41.94 ± 12.78 kg/m^2 (morbid obesity).

Table 1. Patient characteristics (N=17)

Patient Characteristics (N=17)	All Participants	Active Group	Control Group
Age, mean years (± SD)	62±12.51	57±13.54	67±11.39
Age, range years	28-81	28-71	42-81
Gender:			
Male		5	5
Female		4	3
Race:			
Caucasian		7	7
African American		2	1
Other		0	0
Education:			
High School Graduate		4	2
Some College		2	3
University Graduate		3	2
Graduate or Professional Training		0	1
Annual Household Income:			
< $10,000		3	0
$10,000-$24,999		3	4
$25,000-$44,999		2	1
> $45,000		1	3
BMI, kg/m^2, mean (±SD)	41.94±12.78	42.38±8.06	43.56±16.65

Serum CRP Concentration Data:

There was no statistically significant difference in CRP change scores from 0 to 56 days between the Control Group (0.02 ± 1.86 mg/L) and the Active Group (-0.21 ± 0.50 mg/L) (p=.73) (Table 1, Table 2, Figure 2), but a moderate effect size was observed in the Active Group after 56 days of EPA+DHA supplements (.42). In contrast, essentially no effect was observed in the Control Group (.01).

Table 1. CRP Change Scores (0-56) days for Control Group and Active Group Participants

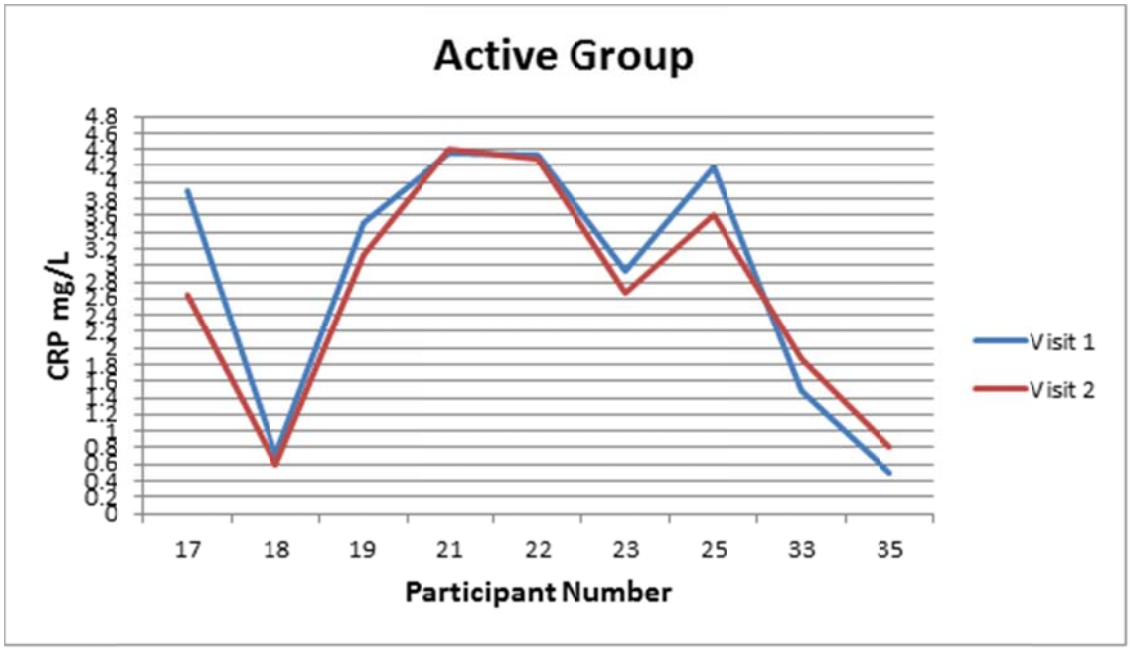
Summary statistics – CRP values at Visit and Visit 2 for Active and Control Groups

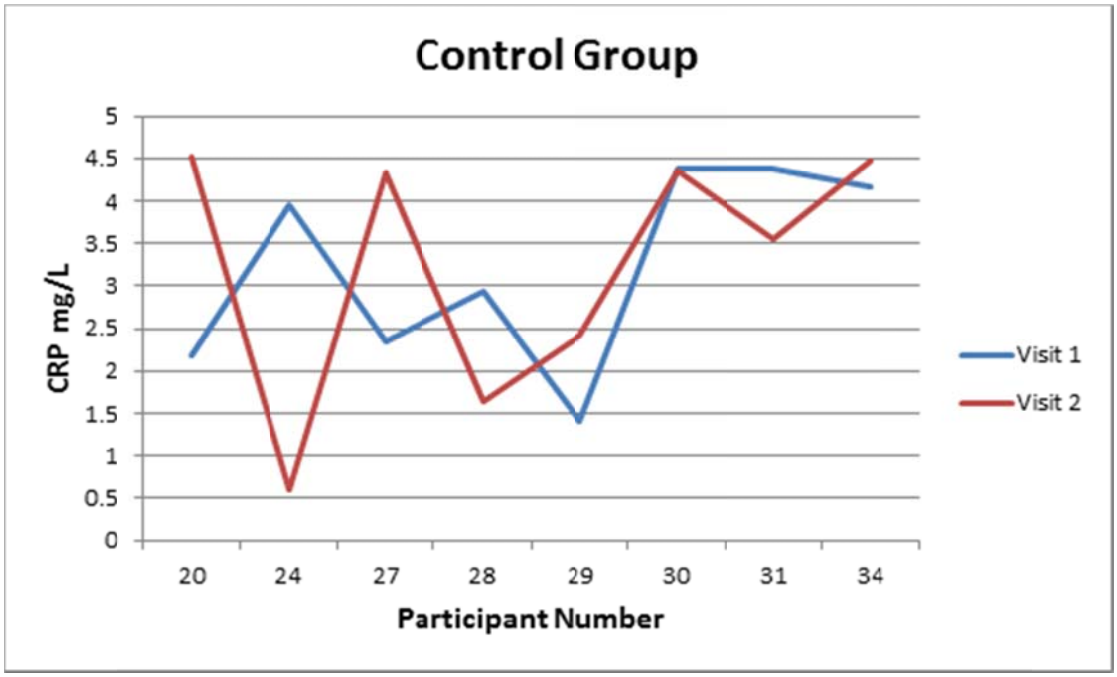
	Group							
	Active				Control			
	n	*Mean*	*STD*	*Median*	*n*	*Mean*	*STD*	*Median*
visit1	9	2.87	1.57	3.51	8	3.22	1.16	3.44
Visit2	9	2.67	1.37	2.66	8	3.23	1.51	3.93
change	9	0.21	0.50	0.11	8	-0.02	1.86	-0.14

T test of a difference in the means of the change scores

			Control change		Active change			
Variable	***Sample Size***	***Overall Mean (STD)***	***N***	***Mean (STD)***	***N***	***Mean (STD)***	***Difference Between Means (95% CI)***	***p Value***	***Effect Size (Cohen's d)***
CHANGE	*17*	*0.1025 (1.2843)*	*8*	*-0.016 (1.8591)*	*9*	*0.2079 (0.4978)*	*-0.2239 (-1.5921, 1.1443)*	*0.73*	*0.1695*

Figure 2. CRP Change Scores (0-56) days for Active and Control Group Participants





V. Summary, Conclusion, and Implications

Summary of Findings

The primary purpose of this two-group, randomized pilot study was to assess the effects of EPA+DHA on systemic inflammation, as determined by CRP levels, in a sample of adults with CVLUs. The data revealed no statistically significant difference in CRP change scores from baseline to 56 days between the Control Group and Active Group; however, there was a moderate effect observed in the Active Group after 56 days of consuming the EPA+DHA supplements. No effect was observed in the Control Group. These findings suggest that the supplements may have reduced CRP levels, which are similar to reports from previous studies (Saifullah et al., 2007 & Bowden, Wilson, Deike, & Gentile, 2009). The supplement effect noted in the current study may have been more pronounced in a larger sample and thus these data may be used to guide future study designs.

The current study data also indicate that the majority of participants were morbidly obese, as evident by a group mean BMI of 41.94 ± 12.78 kg/m^2. We report no significant correlation between BMIs and CRP levels, although other studies have reported a positive link that may be due to an increased production/secretion of proinflammatory cytokines by adipocytes (Tchernof, Nolan, Sites, Ades, & Poehlman, 2002; Visser, Bouter, McQuillan, Wener, & Harris, 1999). Although a positive correlation between BMI and CRP levels was not detected in the current study, the sample's high mean BMI, indicating morbid obesity, increases the risk for other comorbidities such as CVD and diabetes (Narayan, Boyle, Thompson, Gregg, & Williamson, 2007; Ni Mhurch et al., 2004), which are both risk factors for wound healing complications. Higher BMIs are associated with lower levels of adiponectin, a plasma protein (Ai et al., 2011), that has cardioprotective mechanisms, such as "inhibition of pro-inflammatory and hypertrophic

responses and stimulation of endothelial cell responses" (Shibata, Ouchi, Murohara, 2009, p. 608). Collectively, CVD and diabetes compromise circulation of blood and oxygen to the extremities which can lead to local tissue inflammation, tissue breakdown and an increased risk for wound healing delays (Chen & Rogers, 2007).

Finally, this study reports that more than half of the participants (10 of 17) self-reported an income of less than $19,999/year for a 2-person household. According to the U.S. Department of Health and Human Services the 2015 poverty guideline for the 48 contiguous states and the District of Columbia is $15, 930 for a 2-person household. Limited financial resources could be impacting the ability of some patients with chronic wounds such as CVLUs to purchase nutrient dense foods that are essential for efficient wound healing.

Conclusions

In summary, even though no statistically significant difference was noted in CRP change scores from 0 to 56 days between the Control Group and the Active Group, in this small sample, the effect size calculations (.42) suggest that EPA+DHA supplements may have reduced CRP levels and thus inflammation in the Active Group. However, additional studies evaluating the effect of EPA+DHA on CRP levels and wound healing are needed using a larger, more diverse sample of patients with CVLUs. Additionally, the high group mean BMI indicating morbid obesity could increase risk for developing/exacerbating comorbidities associated with CVLUs. Finally, the study findings suggest that a nutrient rich diet may have been out of reach for some of the participants because the majority self-reported an income below the federal poverty guideline. Therefore, limited financial resources could be indirectly contributing to the problem of CVLUs.

Implications for Nursing Practice

An interdisciplinary team that includes nurses and registered dieticians can help provide holistic care for patients with CVLUs. It may be important to assess the diets of CVLU patients at their initial visit to a wound clinic, formulate a plan to address potential nutritional needs, and consider financial barriers to purchasing healthy foods. Additionally, it may be important to assess plasma levels of EPA+DHA and CRP to determine if an oral supplement targeting high inflammation levels is indicated. Interventions that encourage healthy food choices (e.g. fish containing high levels of EPA+DHA, fruits and vegetables) and exercise as tolerated to reach healthy weights may improve healing outcomes and the overall health of CVLU patients. Nurses play an important role in planning and managing patient care; therefore, they can contribute to the healthcare team that delivers the most current evidence-based care to patients with chronic wounds such as CVLUs.

Recommendations

Recommending EPA+DHA supplements, when indicated, could potentially reduce inflammation that may facilitate CVLU healing, but additional research using larger samples of more diverse populations is needed. Future studies evaluating the effects of EPA+DHA on inflammation could also include patients with other types of chronic wounds, such as diabetic foot ulcers and pressure ulcers because chronic inflammation plays a role in the pathogenesis of these wound types too. Finally, the current study used a small sample of participants from a larger study that was not yet completed; therefore, the plasma samples for the current study participants were not available for PUFA quantification. Future studies testing EPA+DHA interventions for wound healing should evaluate the association between PUFA levels and CRP

levels to determine if the supplements were taken correctly and if potential changes in CRP levels were correlated with changes in PUFA levels over time and faster wound healing.

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